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Full Length Research Paper

An Assessment of the Preservative Potentials of Soursop *(Annonamuricata)* Seed Oil on Plantain and Orange Juice.

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An assessment of the preservative potentials of seed oil of soursop *(Annonamuricata)* plant grown in the tropics of West Africa were studied. The seed oil was extracted via Soxhlet extraction method, matured unripe plantain (stage one) selected, while juice was extracted from oranges. Unripe plantain sourced was coated with the extracted oil and orange juice produced was equally treated with 0.3 to 1.5mls of the extracted oil. Phytochemical component and proximate composition of the seed oil, pH and brix of coated plantain, and microbial load of orange juice were determined using standard methods. Results showed antioxidant values to be total soluble phenols (632.91mg/100g), total carotenoid (344.15µg/100g), concentration of tocopherol (360.0µg/100g), ascorbic acid (0.21mg/100g), flavonoid (150.45mg/kg), and anthocyanins (0.01%), while the moisture, ash, crude fat, crude protein, crude fiber and carbohydrate contents were: 52.12%, 1.61%, 5.19%, 9.71%, 1.33% and 30.05% respectively. Microbial load increase of the orange juice was delayed for 3 weeks when compared to control. It has high value for both the bacteria and fungi counts (2.48 log cfu/g and 1.59 log cfu/g respectively), and treated, 1.89 log cfu/g and 0.08 log cfu/g respectively. The oil coating delayed ripening and spoilage of the plantain samples by about 10days.

Keywords: Antioxidant properties, soursop seed oil, microbial load, proximate composition, shelf life, coating.

INTRODUCTION

Human food sources are mainly from plants and animals. Plants, apart from being food to human, they are equally an important sources of medicine for treating ailments (Jyothi *et al*., 2011), and according to history, culture and some other reasons, folk medicine has taken a very important position in many countries, most especially, developing nations of the world (Gandhiraja *et al*., 2009; Gajalakshmi *et al*., 2012). Soursop (*Annona*muricata), also known as “sir sak” or “guanabana”, is a popular fruit tree cultivated in the tropical regions of the world. It is native to tropical North and South America and belongs to the genus **Annona** of the family **Annonaceae,** which includes about 100 species of trees or shrubs (Fasakin *et al.,* 2008). There are about 60 or more species of the genus *Annona*

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family, *Annonaceae*. However, from the entire species, soursop is the most tropical and best to be preserved and processed. Meanwhile, recent studies have shown that growing of the fruits is limited in many countries to certain seasons and localities, because the fruit does not travel much and rarely available fresh for consumption in the areas where they were being grown. Soursop (*Annonamuricata*) is a sweet type of fruit, rich in vitamin C, and as it is or being viewed, it is among the unpopular and underutilized seedy fruits. Its massive disposal of biomass wastes (skin, pulp, seeds etc.) that always occur in most fruit processing units and underutilization of the products has become noticed in the fruit in recent years (Federici *et al.,* 2009). Some research work had been done on the pulp, seed and leaves of the fruit, with some vital acetogenins detected (Gandhiraja *et al.*, 2009; Gajalakshmi *et al.*, 2012). These compounds were found to show some biological, phytochemical or pharmacological activities, such as cytotoxic, antitumour, antimicrobial, antioxidant, pesticide properties etc. (Gajalakshmi *et al.*, 2012; Stone, 1970; Vieira *et al.*, 2010; Pathak *et al.*, 2010; Chukwuka *et al.*, 2011).

Plantains are mostly consumed as vegetable by many people in Nigeria either as raw (ripe one), boiled, roasted or fried with rice or beans, while matured green plantain are boiled and eaten with vegetables or processed into flour for other uses. Most times, peel colour is usually an indicator of the ripeness as the cholorophll content in the peel reduces with ripening (Li *et al.,* 1997), which is a colour change from green to yellow (Marriot *et al.*, 1981). All these are human style of monitoring, but cannot measure the internal parameter of the samples.

The antioxidant profiles of soursop fruit have not really been investigated in respect to their use as preservatives for the prevention or delay of microbial growth in food products and as fruit or vegetable coatings to delay ripening. This research work tend to look at the oil extracted from soursop seed with the intension of it being used as fruit and vegetable coatings to delay ripening processes and protect the fruit and vegetable from water loss and spoilage, as well as enhancing shelf life stability.

MATERIALS AND METHODS

The soursop fruits used for this work were obtained from a soursop tree at Ikare town in Ondo State, Nigeria. Unripe plantains and matured oranges used for the research work were obtained from a local farm within Ilorin metropolis.

Preparation of Seed

The soursop fruits sourced were properly washed, peeled and the pulps squeezed out to remove the seeds that were then dried at room temperature.

Extraction of Oil Using Soxhlet Extractor

A 250-ml Soxhlet extractor apparatus with n-hexane (solvent) was used for extraction work. A known weight of the seed flour was measured into a muslin cloth placed in a thimble of the apparatus. A reactor with a known volume of n-hexane was used with a condenser according to the method of Bokhari *et al.,* 2012.

Procedure for Coating

Matured unripe plantain at stage one of ripeness with uniform colour, size, appearance, and absence of physical defects were selected. The oil extracted were used to coat the skin of the unripen plantain at a thickness of 0.001mm. It was done with aid of a brush dipped into the oil and smeared or rub on the skin of the selected plantain. The coated plantains were spread on a sack in a ventilated room, as well as the control plantain (those without coating) sample under same condition. The rate of ripening was being observed on a daily basis.

Procedure for Orange Juice Production

The production of orange juice involves selection of the orange fruits, washing, peeling, slicing, extracting of juice, sieving, homogenizing, pasteurizing and cooling.

Orange fruits
↓
Washing
↓
Peeling
↓
Slicing
↓
Extraction of juice
↓
Sieving,
↓
Homogenizing
↓
Pasteurizing
↓
Cooling
↓
Orange juice

Figure 1: Production Flowchart of Orange Juice

RESULTS AND DISCUSSION

Physical and Proximate Analysis of Soursop Seed

The physical and proximate composition analyses carried out on the seed of soursop showed that the seed was very rich in oil. The oil was light coloured in appearance and did not solidify at room temperature. It was said to be unsaturated with low fat content of 5.19%. The presence of saturated oil content in the seed could make it susceptible to rancidity. The fat content in the seed was similar to that reported by Nzekwe and Nzekwe, 2011 (5.04%) for another specie of soursop, while Antia *et al.,* 2006 said that dietary fat increases the palatability of food by absorbing and retaining flavours.

Moisture content of the seed was reported to be high on wet basis (52.12%). However, Onimawo, 2002 and Nzekwe and Nzekwe, 2011 respectively reported sharp decrease in the moisture content of soursop seed (8.5% and 2.17%).

Cabohydrate is a potential source of energy and the high quantity in the soursop seed contributes to the energy value in *Annonamuricata*. The seed of *Annonamuricata* had 30.05% carbohydrate value, which was lower to that reported by Onimawo 2002 (47.0%), but higher than that reported by Salami, 2016 (19.14%) for Serendipity berry(*Dioscoreophyllumcumminsii*) seed.

The ash content of any sample is a measure of the quantity of mineral in the sample. The ash content of the soursop seed was 1.61%, which is a measure of the mineral content in the seed. The value obtained was slightly higher than that reported by Sarmento *et al.,* 2015 (1.31%), and lower than that reported by Fasakin *et al.,* 2008 (2.29%) for some other varieties of soursop seed. This variation could be attributed to varietal differences and impact of type and composition of soil of fruit origin (Hidden, 2016). Also, soursop (*Annonamuricata*) seed could actually be said to be high in ash content when compared to that of other seeds such as that of noni (Morindacitrifolia Linn.) (0.93%) as reported by Sarmento *et al.,* 2015, but lower than that of cashew nut (*Anacardiumoccidentale L.*) (2.6%); Pecan (*Caryaillinoinensis*) (2.1%) (Taco, 2011) etc. As a result of this, it could then be said that the seeds contain good quantity of mineral elements, and may then be good food fortificant or useful raw material for animal feed formulation and production of fertilizer.

The fibre content of the soursop seed was 1.33%. It could be recalled that the presence of fibre in seed indicates ability to aid digestion process and prevent the absorption of excess cholesterol in the body (Mensah *et al.,* 2008). The substantial amount of fibre in the seed oil of soursop showed they can help in keeping the digestive system healthy and functioning properly.

The crude protein content of the seed was 9.71%. The value, being slightly high, could make the seed flour a good raw material in the production of animal feeds because of the rich quantity of protein. The protein value was similar to that reported by Sarmento *et al.,* 2015 (8.90%) for *Ximeniaamericana L,* while Pereira *et al.,* (2013) reported (4.24%) for yellow guava, *Psidiumcattleyanum, guabiroba* (5.53%) for *Campomanesiaxanthocarpa* and mandacaru (4.05%) for *Cereus hildmannianus*. Roesler *et al.,* 2007 also reported high protein content for wild plum seeds; seeds of banha (2.7%), cagaita (4.42%), but lower than pequi almond (25.27%), and gold flax seeds (21.6%) (Barroso *et al*., 2014; Lima *et al.,* 2007). The last three being far higher than the value recorded for soursop seed.

Table 2 presents the result of the coated and uncoated plantain samples monitored over twelve days for ripeness and eventual spoilage. It could be seen from the result that after the ten days, all (100%) the uncoated plantain had ripened, while only ten percent (10%) of the coated plantain samples were ripe. Also, forty percent (40%) of the uncoated fully ripened samples were completely spoilt, while only seven percent (7%) of the coated samples were bad under same ambient condition the samples were placed. In other to truly ascertain the preservative potentials of the soursop seed oil, the pH and brix value of the coated and uncoated samples were measured over the twelve days.

It was noticed from Table 3 that the pH value of the coated and uncoated plantain samples ranged from 4.87-5.02 and 4.87-6.32 respectively. The high pH value (6.32) recorded for the uncoated samples after twelve days depicted less acidity and sweetness, with the values increasing from 4.87 to 6.32, noting that the pH values of the coated and uncoated plantain on the first day were the same. The Brix value of the coated and uncoated plantain ranged from 0.02^{o}Brix to 0.44^{o}Brix and 0.02^{o}Brix to 5.90^{o}Brix respectively. The very low brix value recorded for the unripe plantain was normal, as the plantain were at the green stage. It could then be said that the starch in the plantain at the unripe stage was being converted to sugars, as reflected by the brix value. To buttress this, Jamaludin *et al*., 2014 reported that the soluble solids content of banana increased as it reached the ripening stage. The soluble solids content was increasing as the banana was going from unripe to ripe and then overripe stage. Soluble solids are sugars and during ripening, starch contents are converted into sugar and thus, soluble solid of the plantain was increasing as the plantain was going to the ripening stage, and soluble solids are an important trait of hydrolysis of starch into soluble sugars such as glucose, sucrose and fructose according to Marriot *et al*., 1981.

Judging by the outcome of soursop seed oil on the samples, it could be said to have corroborated the report of Raybaudi-Massilia *et al.,* 2015 that states that seed oils normally have antimicrobial property. The lower pH and/or insignificant or negligible changes in the brix value could be due to the effectiveness of the oil coating on the plantain, delaying the period of ripening and eventual spoilage. The soursop seed oil could then be said to have significant effect on the rate of ripening and spoilage, which

Table 1: *Proximate Composition on Soursop Seeds*

Analysis	Percentage (%)
Moisture Content	52.12 ± 0.03
Crude Protein	9.71 ± 0.01
Crude Fiber	1.33 ± 0.01
Crude Fat	5.19 ± 0.01
Total Ash	1.61 ± 0.01
Carbohydrate	30.05 ± 0.03

Mean ± Standard Deviation, ($p \leq 0.05$)

Table 2: Result of the Measurement of the Ripening and Spoilage Level of Coated and Uncoated Plantain Samples

Days	Sample code	Ripened (%)	Spoilage (%)
0	UCP	0.00	0.00
	CP	0.00	0.00
1	UCP	0.00	0.00
	CP	0.00	0.00
2	UCP	0.00	0.00
	CP	0.00	0.00
3	UCP	10.00	0.00
	CP	0.00	0.00
4	UCP	35.00	0.00
	CP	0.00	0.00
5	UCP	65.00	5.00
	CP	00.00	0.00
6	UCP	75.00	15.00
	CP	5.00	0.00
7	UCP	80.00	20.00
	CP	5.00	0.00
8	UCP	85.00	25.00
	CP	7.00	5.00

Table 2: Continue

9		UCP	100.00	30.00
CP	7.00		7.00	
10		UCP	100.00	40.00
CP	10.00	7.00		

Key: UCP = uncoated plantain, CP = coated plantain using Soursop (*Annonamuricata*) seed oil.

Table 3: Chemical Properties of Coated and Uncoated Plantain (pH and Brix)

Days	Analysis	Coated	Uncoated
0	pH	4.87	4.87
	Brix	0.02 ^{o}Bx	0.02 ^{o}Bx
3	pH	4.88	4.96
	Brix	0.02 ^{o}Bx	0.12 ^{o}Bx
5	pH	4.87	5.88
	Brix	0.02 ^{o}Bx	2.40 ^{o}Bx
7	pH	4.89	6.02
	Brix	0.06 ^{o}Bx	4.38 ^{o}Bx
10	pH	4.91	6.24
	Brix	0.11 ^{o}Bx	5.66 ^{o}Bx
12	pH	5.02	6.32
	Brix	0.44 ^{o}Bx	5.90 ^{o}Bx

Key: ^{o}Bx = Brix

may then be attributed to the antioxidant properties of the seed oil of the soursop fruit. As a result of this, it could then be said that the seed oil may be used to delay ripening and spoilage of freshly cut fruits

Microbiological Analysis of the Treated and Untreated Orange Juice during Storage

The microbiological analysis carried out on the orange juice treated with soursop seed oil was monitored over 4 weeks during storage(Figures 2 and 3 respectively), with the colonies counted in unit per gram (log cfu/g). It was observed that the bacterial counts were higher than the fungi counts. The total microbial counts for the samples were very high by the first week, but those on the potato agar media were low. The decrease noticed in the microbial loads of the treated samples at the end of the storage period was from 1.89 log cfu/g to 0.08log cfu/g, while the control sample increased from 1.12 log cfu/g to 2.48log cfu/g over same period. The reduction noticed in the microbial load of the treated samples could be attributed to the action of the soursop seed oil extract added.

The values obtained were within the safe limit for juices, as they had not exceeded the standard values of 1.0 x 10^{4}cfu/ml stated by Ihekoronye (1985) for juice to be safe. Bacterial count is the most predominant and most important organisms in orange juice spoilage. The

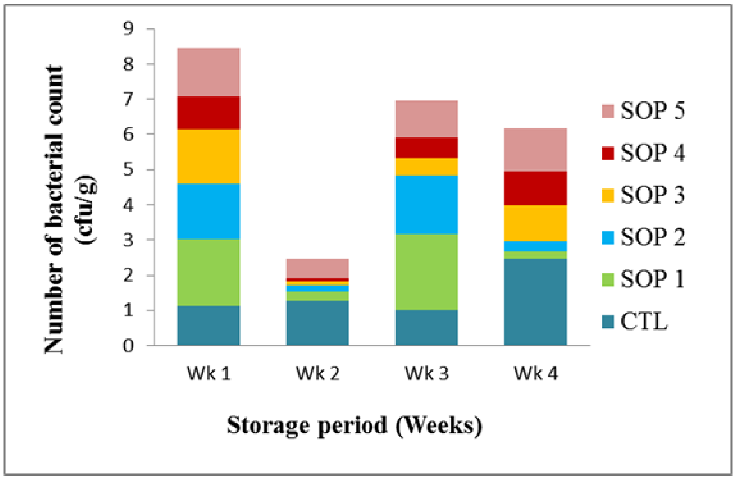

Figure 2: Bacterial count of treated and untreated orange juice during storage
Key: SPO 5 = Orange Juice treated with 1.5ml of Soursop Seed Oil, SPO 4 = Orange Juice treated with 1.2ml of Soursop Seed Oil, SPO 3 = Orange Juice treated with 0.9ml of Soursop Seed Oil, SPO 2 = Orange Juice treated with 0.6ml of Soursop Seed Oil, SPO 1 = Orange Juice treated with 0.3ml of Soursop Seed Oil, CTL = Control-Pure orange juice.

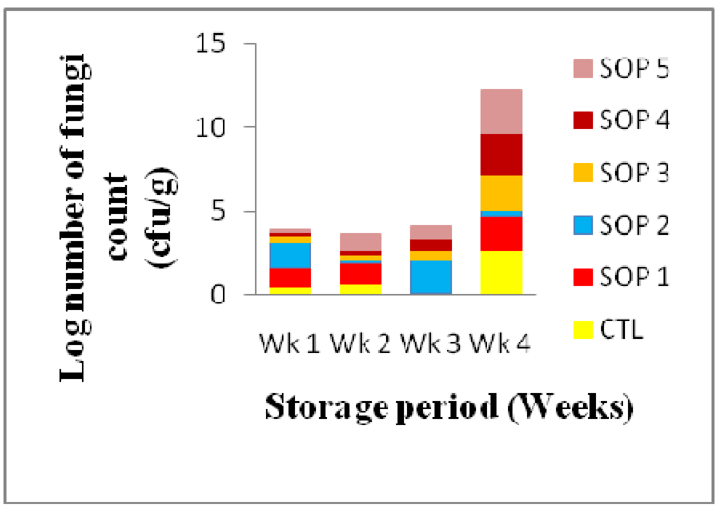

Figure 3: Fungi count of treated and untreated orange juice during storage
Key: SPO 5 = Orange Juice treated with 1.5ml of Soursop Seed Oil, SPO 4 = Orange Juice treated with 1.2ml of Soursop Seed Oil, SPO 3 = Orange Juice treated with 0.9ml of Soursop Seed Oil, SPO 2 = Orange Juice treated with 0.6ml of Soursop Seed Oil, SPO 1 = Orange Juice treated with 0.3ml of Soursop Seed Oil, CTL = Control-Pure of orange juice.

microbial growth curve of sample SOP 2 in Figures2 and 3 demonstrated normal growth curve of micro-organism, similar to that reported by Agoreyo *et al.,* 2003. The organism after adapting for the first two weeks entered an exponential phase by week 3 and then the death phase on week 4 with gradual reduction in numbers. This might be due to the depletion of the nutrients of the orange juice as the nutrients could have been used up by the organisms during these periods, hence resulting in higher mortality rate and struggle for survival (Oyewole, 2012).

Antioxidant Analysis of Soursop Seed Oil

The result of the antioxidant analysis carried out on soursop seed oil is as shown in Table 4. The soursop seed oil had 150.45mg/kg flavonoids, which was similar to the findings on some other varieties of soursop by Onyechi

Table 4:Antioxidant properties of Soursop Seed Oil

Parameter	
Carotenoid µg/100g	344.15 ± 22.14
Ascorbic acid mg/100g	0.21 ± 0.0001
Tocoferol µg/100g	5.93 ± 0.08
Flavonoid mg/kg	150.45 ± 0.92
Phenolic mg/100g	632.91 ±731.75
Anthocyanin %	0.01 ± 0.003

Mean ± Standard deviations

et al., (2012). Flavanoids are known for their antioxidant and antimicrobial activity as reported by Dembitsky *et al.*, 2011. Phenolic contents of soursop seed oil was 632.91mg/100g. This value was higher than4.43g/kg (0.443g/100g)reported by Neuza *et al.,* 2016 for orange seed oil. The value confirmed thatsoursop seed oil is very rich in phenolic compound, and from literature, it has been reported that phenolic compound is a characteristic or property of anticancer (Pieme *et al.*, 2014; Yang *et al.*, 2015). Moreno and Jorge, 2012 reported a high quantity of antioxidant in soursop, most especially the phenolic compounds, which was corroborated by Gutierrez-Abejon *et al.*, 2004.Total anthocyanin content was reported to be 0.01%, followed by ascorbic acids (0.21 mg/100g). Ascorbic acids are antioxidants, as well as a precursor of important vitamin C, showing the soursop seed oil to be of good quality.

Soursop seed oil has total carotenoid content of 344.15µg/100g as seen in Table 4, a value higher than that reported by Neuza *et al.,* 2016 (19.01 mg/kg). Carotenoids are antioxidants as well as a precursor of vitamin A, which confirms soursop seed oil to be of good quality. The tocoferol content of soursop seed oil was 5.93 µg/100g. This was lower to that reported by Neuza*et al.,* 2016 (135.65 mg/kg). Tocoferols are equally antioxidants, as well as a precursor of vitamin E, a property that makes the seed flour to be of good quality. Antioxidant activity generally and especially that of soursop, could be beneficial in human health, as it has the ability to prevent the activity of other chemical compounds called radicals that have the ability to cause damage to cells, including damage that may lead to cancer (NCI, 2014).

It has also been reported by Pathak *et al.*, 2010 and Vijayameena *et al.*, 2013 that soursop has phytochemicals that can exhibit antimicrobial properties, useful in bacterial infection treatment, thus complimenting the outcome of this research work.

CONCLUSIONS

This study showed that the use of natural extract of soursop seed oil containing antioxidant compounds to preserve agricultural produce, could be a cheaper, efficient and effective way of extending the shelf life of fresh and freshly-cut fruits and vegetables. It could be said particularly that the use of soursop (*Annonamuricata*) seed oil on orange juice and plantain as preservative agents, minimized microbial load with its moderate use and delayed ripening by about 10 days respectively, as some of the phytochemical components of the soursop seed oil that were determined confirmed the presence of antioxidants. Hence applying edible coating containing antioxidants to freshly-cut fruits could effectively reduce browning, while increasing the antioxidant capacity of the coated or packed food (Kaliana *et al.,* 2014). Also, the high proximate composition of the soursop seeds confirmed its high quality, which could make it a good raw material for animal feed.

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